	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	2259	igf-1	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:42			0
2	BRS	L2	4478	low adj salt	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:42			0
3	BRS	L3	4	1 same 2	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:42			0
4	BRS	L4	620	"250" adj mg/ml	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:43			0
5	BRS	L5	836	"500" adj mg/ml	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:44			0
6	BRS	L6	4	1 same syrup	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:46			0
7	BRS	L7	4	1 same (5 or 6)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:47			0
8	BRS	L8	3	1 same (5 or 4)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:47			0
9	BRS	L9	1	1 same concentration same density same viscosity	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:48			0

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
10	BRS	L10	14	1 same microsphere	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:48			0
11	BRS	L11	2	1 same microsphere same plga	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:49			0
12	BRS	L12	125	1 same kit	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:51			0
13	BRS	L13	0	1 same kit same (4 or 5)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:51			0
14	BRS	L14	12	shirley adj bret.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:52			0
15	BRS	L15	18	hora adj maninder.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:52			0
16	BRS	L16	17	ohagan adj derek.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:53			0
17	BRS	L17	3	(14 or 15 or 16) and 1	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:53			0

> d his

(FILE 'HOME' ENTERED AT 11:22:30 ON 16 APR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
11:23:01 ON 16 APR 2003

L1 20726 S IGF-1
L2 15761 S LOW SALT
L3 0 S L1 (P) L2
L4 0 S (250 MG/ML)
L5 1 S 250 MG PER ML
L6 1 S 250 (W) MG (W) PER (W) ML
L7 0 S L1 (P) (L5 OR L6)
L8 25374 S PH (W) 5.0
L9 2 S L8 (P) L1
L10 1 DUPLICATE REMOVE L9 (1 DUPLICATE REMOVED)
L11 0 S L1 (P) SYRUP
L12 0 S L1 (P) CONCENTRATION (P) DENSITY (P) VISCOSITY
L13 156 S L1 (P) PH
L14 4 S L13 (P) SALT
L15 4 DUPLICATE REMOVE L14 (0 DUPLICATES REMOVED)
L16 4632 S PLGA
L17 2000 S L16 (P) MICROSPHERE
L18 9 S L1 (P) L17
L19 3 DUPLICATE REMOVE L18 (6 DUPLICATES REMOVED)
L20 104207 S KIT
L21 121 S L20 (P) L1
L22 0 S L21 (P) SYRUP

```
FILE 'HOME' ENTERED AT 11:22:30 ON 16 APR 2003

=> file medline caplus biosis embase scisearch agricola
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 11:23:01 ON 16 APR 2003

FILE 'CAPLUS' ENTERED AT 11:23:01 ON 16 APR 2003
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2003 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 11:23:01 ON 16 APR 2003
COPYRIGHT (C) 2003 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'EMBASE' ENTERED AT 11:23:01 ON 16 APR 2003
COPYRIGHT (C) 2003 Elsevier Science B.V.  All rights reserved.

FILE 'SCISEARCH' ENTERED AT 11:23:01 ON 16 APR 2003
COPYRIGHT (C) 2003 Institute for Scientific Information (ISI) (R)

FILE 'AGRICOLA' ENTERED AT 11:23:01 ON 16 APR 2003

=> s igf-1
L1        20726 IGF-1

=> s low salt
L2        15761 LOW SALT

=> s l1 (p0 l2
MISSING OPERATOR 'L7 (P0'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l1 (p) l2
L3            0 L1 (P) L2

=> s (250 mg/ml)
'ML' IS NOT A VALID FIELD CODE
'ML' IS NOT A VALID FIELD CODE
'ML' IS NOT A VALID FIELD CODE
'ML' IS NOT A VALID FIELD CODE
L4            0 (250 MG/ML)

=> s 250 mg per ml
L5            1 250 MG PER ML

=> s 250 (w) mg (w) per (w) ml
L6            1 250 (W) MG (W) PER (W) ML

=> s l1 (p) (l5 or l6)
L7            0 L1 (P) (L5 OR L6)

=> s ph (w) 5.0
L8        25374 PH (W) 5.0

=> s l8 (p) l1
L9            2 L8 (P) L1

=> duplicate remove l9
DUPLICATE PREFERENCE IS 'MEDLINE, SCISEARCH'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L9
L10           1 DUPLICATE REMOVE L9 (1 DUPLICATE REMOVED)

=> d l10 1 ibib abs

L10  ANSWER 1 OF 1     MEDLINE                          DUPLICATE 1
```

ACCESSION NUMBER: 2001120527 MEDLINE
DOCUMENT NUMBER: 20567455 PubMed ID: 11115387
TITLE: Improved recovery of insulin-like growth factors (IGFs) from bovine colostrum using alkaline diafiltration.
AUTHOR: Hossner K L; Yemm R S
CORPORATE SOURCE: Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523-1171, USA..
khossner@agsci.colostate.edu
SOURCE: BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (2000 Dec) 32 (Pt 3) 161-6.
Journal code: 8609465. ISSN: 0885-4513.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010215

AB Studies to develop a rapid, bioprocess-compatible method to recover low-molecular-mass growth factors from bovine colostrum are reported. Defatted bovine colostrum was subjected to tangential-flow filtration (TFF) using two different filters [polyether sulphone (PES) and regenerated cellulose (RC)] at pH 5.8, pH 8.0 and pH 8.0+0. 01 M NaCl. Single-pass TFF at pH 5.8 using a 100 kDa RC filter resulted in the loss of approx. 90% of insulin-like growth factor I (IGF-I) to non-specific filter adsorption. Comparison of 30 kDa RC and PES filters under single-pass conditions showed that yields of ***IGF*** - ***1*** and IGF-II were highest with RC filters. Yields of IGF-I and protein from both filter types were increased at pH 8.0 and were greatest for the 30 kDa RC filter. Effects of adding large diluent volumes continuously during TFF (diafiltration) were tested at ***pH*** ***5*** . ***0*** and 8.0. The use of 10 diafiltrate vols. at pH 8.0 resulted in the recovery of 15-28% of colostral ***IGF*** - ***1*** from the RC 30 kDa permeates, 2-4-fold greater than under acidic conditions. Yields of IGF-II (39.6%) were unaffected by pH and at least 97% of total protein was retained by the 30 kDa filter at pH 8.0. Denaturing SDS/PAGE analysis of the alkaline RC 30 kDa permeates demonstrated two major regions of stained proteins at 10-13 kDa and 17-19 kDa. Acidic TFF permeates contained additional stained proteins at approximately 90, 48 and 37 kDa. Isoelectric focusing of these samples demonstrated the presence of proteins with isoelectric points of 8.2 and 8.6. The current study demonstrates a one-step bioprocess-compatible technique for the recovery of low-molecular-mass polypeptides from bovine colostrum. By using alkaline diafiltration with RC filters TFF provided optimal recovery of ***IGF*** - ***1*** from colostrum.

```
=> d his

     (FILE 'HOME' ENTERED AT 11:22:30 ON 16 APR 2003)

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
     11:23:01 ON 16 APR 2003
L1         20726 S IGF-1
L2         15761 S LOW SALT
L3             0 S L1 (P) L2
L4             0 S (250 MG/ML)
L5             1 S 250 MG PER ML
L6             1 S 250 (W) MG (W) PER (W) ML
L7             0 S L1 (P) (L5 OR L6)
L8         25374 S PH (W) 5.0
L9             2 S L8 (P) L1
L10            1 DUPLICATE REMOVE L9 (1 DUPLICATE REMOVED)

=> s l1 (p) syrup
L11            0 L1 (P) SYRUP

=> s l1 (p) concentration (p) density (p) viscosity
L12            0 L1 (P) CONCENTRATION (P) DENSITY (P) VISCOSITY

=> s l1 (p) pH
```

```
L13        156 L1 (P) PH

=> s l13 (p) salt
L14          4 L13 (P) SALT

=> duplicate remove l14
PROCESSING COMPLETED FOR L14
L15          4 DUPLICATE REMOVE L14 (0 DUPLICATES REMOVED)

=> d l15 1-4 ibib abs

L15  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2003:76812  CAPLUS
DOCUMENT NUMBER:          138:131557
TITLE:                    Process involving cationic exchange chromatography and
                          hydrophobic interaction chromatograpy for obtaining
                          TGF.beta., IGF-1, lactoperoxidase, and immunoglobulins
                          from milk products
INVENTOR(S):              Kivits, Marinus Gerardus Cornelis; Galama, Catharina
                          Marina; Hendriks, Andor Wilhelm Joseph
PATENT ASSIGNEE(S):       Campina B.V., Neth.; Numico Research B.V.
SOURCE:                   PCT Int. Appl., 26 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
     WO 2003008447      A1   20030130       WO 2002-NL496    20020722
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
             GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
             LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
             PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
             UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU,
             TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
             CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
             NE, SN, TD, TG
PRIORITY APPLN. INFO.:                  EP 2001-202794   A  20010720
                                        EP 2001-202795   A  20010720
AB   The present invention relates to a process for extg. beneficial compds.,
     in particular growth factors, such as TGF .beta. and   ***IGF***  -
       ***1***   from milk. In this process a hydrophobic interaction chromatog.
     step is included. A resin having a Bu group, or a   ***Ph***   group as
     the ligand is used as hydrophobic interaction resin. The resin can be
     eluted with a   ***salt***   gradient which, when the ligand is a
       ***Ph***   group, contains substantially no alc., and thus resulting in
     fractions enriched in the desired growth factors. These fractions can be
     sepd. further by means of a hydroxyapatite column.
REFERENCE COUNT:          6     THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15  ANSWER 2 OF 4  CAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         1998:604690  CAPLUS
DOCUMENT NUMBER:          129:226609
TITLE:                    Refolding of improperly folded polypeptides like
                          recombinant insulin-like growth factor recovered from
                          inclusion bodies
INVENTOR(S):              Builder, Stuart; Hart, Roger; Lester, Philip;
                          Reifsnyder, David
PATENT ASSIGNEE(S):       Genentech, Inc., USA
SOURCE:                   U.S., 38 pp., Cont.-in-part of U.S. Ser. No. 110,664.
                          CODEN: USXXAM
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   2
PATENT INFORMATION:
```

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5808006	A	19980915	US 1994-318628	19941011
US 5663304	A	19970902	US 1993-110664	19930820
WO 9506064	A1	19950302	WO 1994-US9120	19940815

W: CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRIORITY APPLN. INFO.: US 1993-110664 19930820
WO 1994-US9120 19940815

AB A method for solubilizing and refolding peptides manufd. in bacterial hosts and accumulated as inclusion bodies is described. The method is optimized for the recovery of insulin-like growth factor and its analogs and is inexpensive. The polypeptide is resuspended at 0.1-15 mg/mL in a buffer having a ***pH*** of about 7-12 of 5-40 vol./vol.% of an alc. or polar aprotic solvent, about 0.2-3M of an alk. earth, alkali metal, or ammonium ***salt*** , about 0.1-9M of a chaotropic agent, and about 0.10-15 .mu.M of a copper or manganese ***salt*** . The protein is allowed to refold by incubating it in this buffer. The presence of the low concns. of copper or manganese minimizes the formation of incorrectly folded proteins and avoids the need for disulfide exchange agents. The method can also be used in two-phase systems where cell lysates are fractionated by phase partition and the phase contg. the inclusion bodies is under conditions suitable for solubilization and renaturation. The method is demonstrated with ***IGF*** - ***1*** manufd. in Escherichia coli by expression of a cDNA. From a large-scale fermn. (600-800 L) the protein could be refolded with a recovery of .apprx.50%. Expts. using two-phase systems are reported.

REFERENCE COUNT: 112 THERE ARE 112 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:547305 CAPLUS
DOCUMENT NUMBER: 127:149410
TITLE: Preparation of nitrogen heterocyclic peptide analogs as growth-hormone secretagogues
INVENTOR(S): Carpino, Philip A.; Jardine, Dasilva Paul A.; Lefker, Bruce A.; Ragan, John A.
PATENT ASSIGNEE(S): Pfizer Inc., USA; Carpino, Philip A.; Jardine, Dasilva Paul A.; Lefker, Bruce A.; Ragan, John A.
SOURCE: PCT Int. Appl., 152 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9724369	A1	19970710	WO 1996-IB1353	19961204
			W: AU, BG, BR, BY, CA, CN, CZ, HU, IL, IS, JP, KR, KZ, LK, LV, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TR, UA, US, UZ, VN	
			RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG	
TW 432073	B	20010501	TW 1996-85113857	19961113
CA 2241725	AA	19970710	CA 1996-2241725	19961204
AU 9675850	A1	19970728	AU 1996-75850	19961204
AU 716934	B2	20000309		
EP 869968	A1	19981014	EP 1996-938434	19961204
			R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, SI, LV, FI, RO	
CN 1206422	A	19990127	CN 1996-199388	19961204
JP 11501945	T2	19990216	JP 1996-524124	19961204
BR 9612465	A	19990713	BR 1996-12465	19961204
JP 2001213800	A2	20010807	JP 2000-386997	19961204
RU 2172742	C2	20010827	RU 1998-112108	19961204
ZA 9610858	A	19980623	ZA 1996-10858	19961223
NO 9802991	A	19980826	NO 1998-2991	19980626
US 6107306	A	20000822	US 1999-259691	19990301
US 6110932	A	20000829	US 1999-258956	19990301
US 6124264	A	20000926	US 1999-259776	19990301
US 6278000	B1	20010821	US 1999-470668	19991222

US 6306875	B1	2001[redacted]23	US 2000-593582	20000613	
US 6313140	B1	2001[redacted]6	US 2000-593581	20000[redacted]	
US 2002049196	A1	20020425	US 2000-734274	20001211	
US 6482825	B2	20021119			
PRIORITY APPLN. INFO.:			US 1995-9469P	P	19951228
			JP 1997-524124	A3	19961204
			WO 1996-IB1353	W	19961204
			US 1998-68566	A3	19980521
			US 1999-258956	A1	19990301
			US 1999-259691	A1	19990301
			US 1999-259776	A3	19990301

OTHER SOURCE(S): MARPAT 127:149410
GI

/ Structure 1 in file .gra /

AB Title compds. I [X = CH2, bond; X1, X2 = independently bond, CH2, CH2CH2; Y = O, S; R1 = H, CN, side chain such as (un)substituted (CH2)qN(X6)R, (CH2)tA1, etc.; q = 0-4, t = 0=3; X6 = H, (un)substituted C1-6 alkyl, C3-7 cycloalkyl, etc; A1 = (un)substituted C5-7 cycloalkenyl, ***Ph*** , 4-8 membered heterocycle, etc.; R2 = H, (un)substituted C1-8 alkyl, C0-3 alkyl-C3-8 cycloalkyl, C1-4 alkyl-A1; R3 = (un)substituted A1, C1-10 alkyl, C1-6 alkyl-A1, C1-6 alkyl-C3-7 cycloalkyl, etc; R4 = H, (un)substituted C1-6 alkyl, C3-7 cycloalkyl; or R3 and R4 form a ring; X4 = H, C1-6 alkyl; or X4 and R4 form a ring; R6 = bond, Z1(CH2)aC(X5)(X5a)(CH2)b; a = 0-3; b = 0-3; X5, X5a = independently H, CF3, A1, (un)substituted C1-6 alkyl, or form a ring; Z1 = bond, O, NX12, X12 = H, (un)substituted C1-6 alkyl; R7, R8 = independently (un)substituted C1-6 alkyl, or forma a ring] and pharmaceutically-acceptable ***salts*** thereof, are growth hormone secretagogues and increase the level of endogenous growth hormone. These compds. are useful for the treatment and prevention of osteoporosis, congestive heart failure, frailty assocd. with aging, obesity; accelerating bone fracture repair, attenuating protein catabolic response after a major operation, reducing cachexia and protein loss due to chronic illness, accelerating the recovery of burn patients or patients having undergone major surgery; improving muscle strength, mobility, maintenance of skin thickness, metabolic homeostasis or renal homeostasis. These compds. are also useful in treating osteoporosis when used in combination with: a bisphosphonate compd. such as alendronate; estrogen, premarin, and optionally progesterone; an estrogen agonist or antagonist; or calcitonin, and pharmaceutical compns. useful therefor. Further, the present invention is directed to pharmaceutical compns. useful for increasing the endogenous prodn. or release of growth hormone in a human or other animal which comprises an effective amt. of a compd. of the present invention and a growth hormone secretagogue selected from GHRP-6, Hexarelin, GHRP-1, growth hormone releasing factor (GRF), ***IGF*** - ***1*** , IGF-2 or B-HT920. The invention is also directed to intermediates useful in the prepn. of I. Thus, alkylation of oxopiperidinecarboxylate ester II (Boc = Me3CO2C) (prepn. given) with PhCH2Br, followed by cyclocondensation with MeNHNH2 and deprotection gave pyrazolopyridinone III. Amidation of Boc-Aib-D-Ser(CH2Ph)-OH (prepn. given) with III, diastereomer sepn., and deprotection, gave sepd. title compds. IV as their HCl ***salts*** .

L15 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER:	1995:623433 CAPLUS
DOCUMENT NUMBER:	123:27196
TITLE:	Refolding of improperly folded polypeptides like recombinant insulin-like growth factor recovered from inclusion bodies
INVENTOR(S):	Builder, Stuart; Hart, Roger; Lester, Phillip; Reifsnyder, David
PATENT ASSIGNEE(S):	Genetech, Inc., USA
SOURCE:	PCT Int. Appl., 74 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9506064	A1	19950302	WO 1994-US9120	19940815
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5663304	A	19970902	US 1993-110664	19930820
CA 2168552	AA	19950302	CA 1994-2168552	19940815
EP 714406	A1	19960605	EP 1994-927917	19940815
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 09501693	T2	19970218	JP 1994-507626	19940815
US 5808006	A	19980915	US 1994-318628	19941011
US 5756672	A	19980526	US 1995-470108	19950606

PRIORITY APPLN. INFO.: US 1993-110664 19930820
WO 1994-US9120 19940815

AB A method for solubilizing and refolding peptides manufd. in bacterial hosts and accumulated as inclusion bodies is described. The method is optimized for the recovery of insulin-like growth factor and its analogs and is inexpensive. The polypeptide is resuspended at 0.1 - 15 mg/mL in a buffer having a ***pH*** of about 7-12 of 5-40 vol.% of an alc. or polar aprotic solvent, about 0.2 to 3 M of an alk. earth, alkali metal, or ammonium ***salt*** , about 0.1 to 9 M of a chaotropic agent, and about 0.10 to 15 .mu.M of a copper or manganese ***salt*** . The protein is allowed to refold by incubating it in this buffer. The presence of the low concns. of copper or manganese minimizes the formation of incorrectly folded proteins and avoids the need for disulfide exchange agents. The method can also be used in two-phase systems where cell lysates are fractionated by phase partition and the phase contg. the inclusion bodies is under conditions suitable for solubilization and renaturation. The method is demonstrated with ***IGF*** - ***1*** manufd. in Escherichia coli by expression of a cDNA. From a large-scale fermn. (600-800 L) the protein could be refolded with a recovery of about 50%. Expts. using two-phase systems are reported.

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(FILE 'HOME' ENTERED AT 11:22:30 ON 16 APR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 11:23:01 ON 16 APR 2003

```
L1        20726 S IGF-1
L2        15761 S LOW SALT
L3            0 S L1 (P) L2
L4            0 S (250 MG/ML)
L5            1 S 250 MG PER ML
L6            1 S 250 (W) MG (W) PER (W) ML
L7            0 S L1 (P) (L5 OR L6)
L8        25374 S PH (W) 5.0
L9            2 S L8 (P) L1
L10           1 DUPLICATE REMOVE L9 (1 DUPLICATE REMOVED)
L11           0 S L1 (P) SYRUP
L12           0 S L1 (P) CONCENTRATION (P) DENSITY (P) VISCOSITY
L13         156 S L1 (P) PH
L14           4 S L13 (P) SALT
L15           4 DUPLICATE REMOVE L14 (0 DUPLICATES REMOVED)

=> s plga
L16        4632 PLGA

=> s l16 (p) microsphere
L17        2000 L16 (P) MICROSPHERE

=> s l1 (p) l17
L18           9 L1 (P) L17

=> duplicate remove l18
DUPLICATE PREFERENCE IS 'MEDLINE, EMBASE, SCISEARCH'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L18
L19           3 DUPLICATE REMOVE L18 (6 DUPLICATES REMOVED)
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=> d l19 1-3 ibib abs

L19 ANSWER 1 OF 3 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000267100 MEDLINE
DOCUMENT NUMBER: 20267100 PubMed ID: 10809103
TITLE: De novo adipose tissue generation through long-term, local delivery of insulin and insulin-like growth factor-1 by PLGA/PEG microspheres in an in vivo rat model: a novel concept and capability.
AUTHOR: Yuksel E; Weinfeld A B; Cleek R; Waugh J M; Jensen J; Boutros S; Shenaq S M; Spira M
CORPORATE SOURCE: Division of Plastic Surgery at Baylor College of Medicine, Houston, Texas, USA.. eyuksel@bcm.tmc.edu
SOURCE: PLASTIC AND RECONSTRUCTIVE SURGERY, (2000 Apr) 105 (5) 1721-9.
Journal code: 1306050. ISSN: 0032-1052.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000606
Last Updated on STN: 20000606
Entered Medline: 20000519

AB This study was undertaken to characterize the duration of long-term growth factor delivery by poly(lactic-co-glycolic-acid)-polyethylene glycol (***PLGA*** /PEG) ***microspheres*** and to evaluate the potential of long-term delivery of insulin and insulin-like growth factor-1 (***IGF*** - ***1***) for the de novo generation of adipose tissue in vivo. ***PLGA*** /PEG ***microspheres*** containing insulin and ***IGF*** - ***1*** , separately, were produced by a double-emulsion solvent-extraction technique. In the first phase of the experiment, the in vitro release kinetics of the ***microspheres*** were evaluated for the optical density and polyacrylamide gel electrophoresis of solutions incubated with insulin-containing ***microspheres*** for four different periods of time (n = 1). The finding of increased concentrations of soluble insulin with increased incubation time confirmed continual protein release. In the second stage of the experiment, 16 rats were divided equally into four study groups (insulin, ***IGF*** - ***1*** , insulin + ***IGF*** - ***1*** , and blank ***microspheres***) (n = 4). Insulin and ***IGF*** - ***1*** containing ***microspheres*** were administered directly to the deep muscular fascia of the rat abdominal wall to evaluate the potential for de novo adipose tissue generation via adipogenic differentiation from native nonadipocyte cell pools in vivo. Animals treated with blank ***microspheres*** served as an external control group. At the 4-week harvest period, multiple ectopic islands of adipose tissue were observed on the abdominal wall of the animals treated with insulin, ***IGF*** - ***1*** , and insulin + ***IGF*** - ***1*** ***microspheres*** . Such islands were not seen in the blank ***microsphere*** group. Hematoxylin and eosin-stained sections of the growth factor groups demonstrated mature adipocytes interspersed with fibrous tissue superficial to the abdominal wall musculature and continuous with the fascia. Oil-Red-O stained sections demonstrated that these cells contained lipid. Computer-aided image analysis of histologic sections confirmed that there were statistically significant increases in the amount of "ectopic" adipose neotissue developed on the abdominal wall of animals treated with growth factor ***microspheres*** . In conclusion, this study confirms the long-term release of proteins from ***PLGA*** /PEG ***microspheres*** up to 4 weeks and demonstrates the potential of long-term local insulin and ***IGF*** - ***1*** to induce adipogenic differentiation to mature lipid-containing adipocytes from nonadipocyte cell pools in vivo at 4 weeks.

L19 ANSWER 2 OF 3 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000267099 MEDLINE
DOCUMENT NUMBER: 20267099 PubMed ID: 10809102
TITLE: Increased free fat-graft survival with the long-term, local delivery of insulin, insulin-like growth factor-I, and basic fibroblast growth factor by PLGA/PEG microspheres.
AUTHOR: Yuksel E; Weinfeld A B; Cleek R; Wamsley S; Jensen J;

Boutros S; Waugh J M; Shenaq S M; Spira M
CORPORATE SOURCE: Division of Plastic Surgery at Baylor College of Medicine, Houston, Texas, USA.. eyuksel@bcm.tmc.edu
SOURCE: PLASTIC AND RECONSTRUCTIVE SURGERY, (2000 Apr) 105 (5) 1712-20.
Journal code: 1306050. ISSN: 0032-1052.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000606
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AB The present investigation evaluates the effects of long-term, local delivery of insulin, insulin-like growth factor-1 (***IGF*** - ***1***), and basic fibroblast growth factor (bFGF) on fat-graft survival using a poly (lactic-co-glycolic-acid)-polyethylene glycol (***PLGA*** /PEG) ***microsphere*** delivery system. Twelve-micrometer ***PLGA*** /PEG ***microspheres*** incorporated separately with insulin, ***IGF*** - ***1*** , and bFGF were manufactured using a double-emulsion solvent-extraction technique. Inguinal fat from Sprague Dawley rats was harvested, diced, washed, and mixed with (1) insulin ***microspheres*** , (2) insulin-like growth factor-1 ***microspheres*** , (3) basic fibroblast growth factor ***microspheres*** , (4) a combination of the insulin and ***IGF*** - ***1*** ***microspheres*** , and (5) a combination of insulin, ***IGF*** - ***1*** , and bFGF ***microspheres*** . The treated fat grafts were implanted autologously into subdermal pockets in six animals for each group. Animals receiving untreated fat grafts and fat grafts treated with blank ***microspheres*** constituted two external control groups (six animals per external control group). At 12 weeks, all fat-graft groups were compared on the basis of weight maintenance and a histomorphometric analysis of adipocyte area percentage, indices of volume retention and cell composition, respectively. Weight maintenance was defined as the final graft weight as a percent of the implanted graft weight. All growth factor treatments significantly increased fat-graft weight maintenance objectively, and volume maintenance grossly, in comparison with the untreated and blank ***microsphere*** -treated controls. Treatment with insulin and ***IGF*** - ***1*** , alone or in combination, was found to increase the adipocyte area percentage in comparison with fat grafts treated with bFGF alone or in combination with other growth factors. In conclusion, the findings of this study indicate that long-term, local delivery of growth factors with ***PLGA*** /PEG ***microspheres*** has the potential to increase fat-graft survival rates. Further, the type of growth factor delivered may influence the cellular/stromal composition of the grafted tissue.

L19 ANSWER 3 OF 3 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000415295 MEDLINE
DOCUMENT NUMBER: 20401760 PubMed ID: 10946936
TITLE: Augmentation of adipofascial flaps using the long-term local delivery of insulin and insulin-like growth factor-1.
AUTHOR: Yuksel E; Weinfeld A B; Cleek R; Jensen J; Wamsley S; Waugh J M; Spira M; Shenaq S
CORPORATE SOURCE: Division of Plastic Surgery, Baylor College of Medicine, and the Institute of Bioengineering and Bioscience, Rice University, Houston, Texas, USA.
SOURCE: PLASTIC AND RECONSTRUCTIVE SURGERY, (2000 Aug) 106 (2) 373-82.
Journal code: 1306050. ISSN: 0032-1052.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000907
Last Updated on STN: 20000907
Entered Medline: 20000828

AB The adipofascial flaps currently described in the literature frequently lack the volume requirements for reconstructive goals. In this study, the authors examined the use of long-term local delivery of insulin and

insulin-like growth factor-1 (***IGF*** - ***1***) using polylactic-coglycolic acid/[redacted]yethylene glycol (***PLGA***[redacted]PEG) ***microspheres*** to augment inguinal adipofascial flaps based on the inferior epigastric vessels in the rat. Two flap models, the island flap and the limited dissection flap, were used to demonstrate simultaneous treatment and pretreatment modalities, respectively. Experimental groups received 12.5 mg of insulin ***microspheres*** (carrying 1 IU of insulin) plus 12.5 mg of ***IGF*** - ***1*** ***microspheres*** (carrying 2.5 microg of ***IGF*** - ***1***). A group undergoing the operation only (no treatment with ***microspheres***) and a group treated with blank ***microspheres*** (no growth factor) served as external controls for the surgical procedure and the drug delivery device, respectively. In all groups (n = 5 animals in each), the contralateral flap served as an internal control. Upon harvest on postoperative day 28, the insulin and ***IGF*** - ***1*** -treated flaps in both models weighed statistically more than the internal control flaps and the two external control flaps. Likewise, on gross inspection, the adipogenic growth factor-treated flaps had greater volumes than the internal control flap groups and both of the external control flap groups (operation only and blank ***microspheres***). Other intergroup comparisons suggested the absence of a systemic insulin and ***IGF*** - ***1*** effect on adiposity. A histomorphometric analysis suggested (1) that insulin and ***IGF*** - ***1*** treatment does not alter flap cell composition and (2) that flap augmentation is secondary to the stimulation of cell proliferation and adipocytic differentiation rather than the hypertrophy of mature adipocytes. Further evidence in favor of cell proliferation and differentiation was the discovery of nonanatomic, ectopic fat islands on the pedicle sheath of the treated flaps and the lack of variation in cell size distribution among groups. The authors concluded that the long-term local delivery of insulin and ***IGF*** - ***1*** with ***PLGA*** /PEG ***microspheres*** is an effective method of adipofascial flap augmentation; this method increases the number of mature adipocytes rather than increasing the size of preexisting cells.

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=> d his

     (FILE 'HOME' ENTERED AT 11:22:30 ON 16 APR 2003)

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
     11:23:01 ON 16 APR 2003
L1         20726 S IGF-1
L2         15761 S LOW SALT
L3             0 S L1 (P) L2
L4             0 S (250 MG/ML)
L5             1 S 250 MG PER ML
L6             1 S 250 (W) MG (W) PER (W) ML
L7             0 S L1 (P) (L5 OR L6)
L8         25374 S PH (W) 5.0
L9             2 S L8 (P) L1
L10            1 DUPLICATE REMOVE L9 (1 DUPLICATE REMOVED)
L11            0 S L1 (P) SYRUP
L12            0 S L1 (P) CONCENTRATION (P) DENSITY (P) VISCOSITY
L13          156 S L1 (P) PH
L14            4 S L13 (P) SALT
L15            4 DUPLICATE REMOVE L14 (0 DUPLICATES REMOVED)
L16         4632 S PLGA
L17         2000 S L16 (P) MICROSPHERE
L18            9 S L1 (P) L17
L19            3 DUPLICATE REMOVE L18 (6 DUPLICATES REMOVED)

=> s kit
L20      104207 KIT

=> s l20 (p) l1
L21          121 L20 (P) L1

=> s l21 (p) syrup
L22            0 L21 (P) SYRUP

=> d his
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      (FILE 'HOME' ENTERED AT 11:2[redacted]30 ON 16 APR 2003)

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      11:23:01 ON 16 APR 2003
L1         20726 S IGF-1
L2         15761 S LOW SALT
L3             0 S L1 (P) L2
L4             0 S (250 MG/ML)
L5             1 S 250 MG PER ML
L6             1 S 250 (W) MG (W) PER (W) ML
L7             0 S L1 (P) (L5 OR L6)
L8         25374 S PH (W) 5.0
L9             2 S L8 (P) L1
L10            1 DUPLICATE REMOVE L9 (1 DUPLICATE REMOVED)
L11            0 S L1 (P) SYRUP
L12            0 S L1 (P) CONCENTRATION (P) DENSITY (P) VISCOSITY
L13          156 S L1 (P) PH
L14            4 S L13 (P) SALT
L15            4 DUPLICATE REMOVE L14 (0 DUPLICATES REMOVED)
L16         4632 S PLGA
L17         2000 S L16 (P) MICROSPHERE
L18            9 S L1 (P) L17
L19            3 DUPLICATE REMOVE L18 (6 DUPLICATES REMOVED)
L20       104207 S KIT
L21          121 S L20 (P) L1
L22            0 S L21 (P) SYRUP

=> log y
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                  ENTRY    SESSION
FULL ESTIMATED COST                               67.95      68.16

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)      SINCE FILE      TOTAL
                                                  ENTRY    SESSION
CA SUBSCRIBER PRICE                               -2.60      -2.60

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